



US Environmental Protection Agency Office of Pesticide Programs

**Office of Pesticide Programs
Microbiology Laboratory
Environmental Science Center, Ft. Meade, MD**

**Standard Operating Procedure for Use and Maintenance
of Biological Safety Cabinets**

SOP Number: QC-06-04

Date Revised: 06-30-08

EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

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for
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1.0 SCOPE AND APPLICATION:

- 1.1 The purpose of this SOP is to describe the procedure used to monitor the use and maintenance of the Biological Safety Cabinets (BSC). Performing maintenance and repairs on the BSCs and maintaining annual certification of the BSCs are the responsibilities of the Facility Manager or his designees (e.g., operations, maintenance and repair contractors).

2.0 DEFINITIONS:

- 2.1 BSC = biological safety cabinet
- 2.2 FPM = feet per minute
- 2.3 CFM = cubic feet per minute
- 2.4 HEPA = high efficiency particulate air

3.0 HEALTH AND SAFETY:

- 3.1 Laboratory workers are required to read and be familiar with SOP MB-01, Lab Biosafety, prior to working with microorganisms in the BSC. Documentation of training and familiarization with SOP MB-01 can be found in the training file for each employee.
- 3.2 All work involving manipulation of cultures (SOP MB-01, Lab Biosafety) of microorganisms, regardless of biosafety level, is performed in the BSC.

4.0 CAUTIONS:

- 4.1 Failure to clean the ultraviolet lamps in the BSC will reduce the lamps' effectiveness. Periodically clean the ultraviolet lamps in the BSC with a lint-free cloth dampened with alcohol.
- 4.2 Periodic cleaning of the area under the BSC work surface and grill is recommended.

5.0 INTERFERENCES:

- 5.1 Laboratory staff performing work in the BSC must wear a long-sleeved lab coat and are encouraged to wear gloves even when not manipulating culture. This will minimize the shedding of skin flora into the work area and concurrently protect the hands and arms from viable agent contamination (see ref. 15.2, p. 41).

6.0 PERSONNEL QUALIFICATIONS:

- 6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

- 7.1 BSC #1 NuAire Model No. NU-430-600 Serial No. 78536AFU located in room B204
- 7.2 BSC #2 NuAire Model No. NU-430-600 Serial No. 78538AFU located in room B204
- 7.3 BSC #3 NuAire Model No. NU-430-600 Serial No. 78537AFU located in room B204
- 7.4 BSC #4 NuAire Model No. NU-430-600 Serial No. 78539AFU located in room B205
- 7.5 BSC #5 NuAire Model No. NU-430-600 Serial No. 76788AFP located in room B207
- 7.6 BSC #6 NuAire Model No. NU-430-600 Serial No. 76786AFP located in room B207
- 7.7 BSC #7 NuAire Model No. NU-430-600 Serial No. 85756AGY located in room B202
- 7.8 BSC #8 NuAire Model No. NU-430-600 Serial No. 85755AGY located in room B202
- 7.9 BSC #9 NuAire Model No NU 430-600, Serial No. 90496042204 located in room B201
- 7.10 BSC # 10 NuAire Model No. NU 430-600, Serial No. 90474042104 located in room D122

8.0 INSTRUMENT OR METHOD CALIBRATION:

- 8.1 Work zone air downflow is uniform and held to an average velocity of 60 LFPM (0.30 m/s) measured in the plane of the bottom edge of the viewing window. The

BSC will alarm if the average velocity falls below 54 LFPM or above 64 LFPM. The significance of this airflow control is that the work zone air is flowing down like a huge piston carrying contaminants rapidly out of the work zone with 24 air changes per minute. The work zone airflow is ducted such that the quantity of air leaving through the rear perforated area is half of the downflow air quantity. The remainder, that is the other half of the downflow air, and the air entering through the work access opening flow into the front perforated area. Room air flowing into the 8 inch (203 mm) work access opening forms the air barrier protecting personnel at 105 \pm 5 fpm (0.53 \pm 0.25 m/s). The exhaust volume is displayed on the front panel. The BSC will alarm if the exhaust volume falls below 980 CFM or rises to above 1250 CFM.

9.0 SAMPLE HANDLING AND STORAGE: Not applicable

10.0 PROCEDURE AND ANALYSIS:

- 10.1 Turn on the blowers, lights, and outlets, and allow to operate for a minimum of 15 minutes before aseptic manipulations are begun in the BSC.
- 10.2 Disinfect the interior surface of the BSC work space thoroughly by spraying/wetting with an appropriate amount of a hospital disinfectant and allow to stand for the label-specified contact time before wiping.
 - 10.2.1 Halogenated materials (e.g., bleach) are not recommended for use on stainless steel surfaces of the BSC.
- 10.3 Place the apparatus and materials into the cabinet. Care must be exercised that no items be placed over the front intake grills. The work area should be arranged so that clean and dirty (used) materials are well separated. Passage of contaminated materials over uninoculated cultures or clean glassware should be avoided and transfer of viable materials should be performed as deeply into the cabinet (away from open face) as possible.
- 10.4 Additional purging of the work space without any user activity should be allowed for 2-3 minutes after materials and apparatus have been placed in it. This will rid the area of all contamination that may have been introduced with the items.
- 10.5 Record the Downflow (FPM) and Exhaust (CFM) rates on the BSC Monitoring Record Form (see 16.1) immediately prior to use.
- 10.6 Work can now be performed.

- 10.7 Applying a Bunsen burner flame to tube and flask necks is a conventional method of minimizing airborne contamination when the tubes or flasks are opened. Because the laminar flow BSCs create an environment that has little to no airborne particulates, it is not necessary to flame tube and flask necks. Gas burners contribute to heat build-up in the cabinet and disrupt the laminar air flow. If a procedure requires the use of a flame (e.g., to flame sterilize hooks, etc.), use a burner with a pilot light so that the flame can be ignited only when required. Do not use constant flame gas burners. Gas burners should only be used from the center of the work surface to the right rear, where resulting air turbulence will have a minimal effect. Do not use gas burners on the left of the work surface due to its influence on the electronic airflow control system (see ref. 15.2, p. 40).
- 10.8 Once work is completed, allow the BSC to run for a 2-3 minute period without activity to purge the unit. Remove all materials, cultures, and equipment and decontaminate BSC as in 10.2.
- 10.8.1 As an additional step, at the conclusion of activities involving bacteria in spore form (e.g., *Bacillus subtilis* spore suspensions, spore strips, etc.) turn on the ultraviolet light and leave it on overnight.
- 10.8.2 Refer to the laboratory's Biosafety Plan for *Bacillus anthracis* for BSC clean up procedures following work with select agents.
- 10.9 A careful check of grills and diffuser grids should be made for spilled or splashed nutrients which may support microbial growth and result in the liberation of contaminants in the protected work environment.
- 10.10 A permanent paper catch is installed behind the rear divider panel of the work zone. This area forms the return air path to the motor/blower. If the air flow is blocked, it could seriously affect the performance of the cabinet. The paper catch should be checked and cleaned no less than weekly; daily if procedures require the use of paper products. Any paper removed must be properly disposed of as contaminated hazardous waste.
- 10.11 Turn off lights and outlets. Turn off blowers as per facility recommendations. Do not use cabinet as a repository for excess lab equipment during periods of non-operation.
- 10.12 Record maintenance on the BSC Monitoring Record Form (see 16.1).
- 11.0 DATA ANALYSIS/CALCULATIONS: None

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

- 12.1 Data will be recorded promptly, legibly, and in indelible ink on the BSC Monitoring Record Form (see 16.1). Completed forms are archived in notebooks kept in secured file cabinets in file room D217. Only authorized personnel have access to the secured files. Archived data is subject to OPP=s official retention schedule contained in SOP ADM-03 (Records and Archives).

13.0 QUALITY CONTROL:

- 13.1 The BSCs will be recertified annually by a qualified technician. Scheduling recertification is the responsibility of the Facility Manager or his designees. Certificates will be maintained by the Facility Manager.
- 13.2 Under normal usage and barring an accident (puncture), the HEPA filters on each BSC do not need replacement until the exhaust volume or the access flow velocity cannot be maintained. Making this determination and scheduling HEPA filter replacement is the responsibility of the Facility Manager or his designees. Once the HEPA filter is replaced, the BSC will be recertified and the certification will be annotated by a Performance Certification sticker located to the left of the control panel. Certificates will be maintained by the Facility Manager.

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

- 14.1 When laboratory staff believe that service is necessary for the BSCs (e.g., alarms are sounding, the BSC fails to advance past the warm-up mode, the BSC is not functioning as it usually does, etc.), they will notify the Facilities Helpline at (410) 305-4357. The Facility Manager or his designees will investigate and make a service call if necessary. Laboratory staff will note the event on the BSC Monitoring Record Form (16.1) as well as any corrective action taken.

15.0 REFERENCES:

- 15.1 Bordner, R.H., Winter, J.A. &, Scarpino, P.V., eds. 1978. Microbiological Methods for Monitoring the Environment, Water and Wastes. EPA 600/8-78-017, Environmental Monitoring & Support Lab., U.S. Environmental Protection Agency, Cincinnati, Ohio.
- 15.2 NuAire Labguard Class II, Type B2 Laminar Flow Biological Safety Cabinet Operation and Maintenance Manual.

16.0 FORMS AND DATA SHEETS:

16.1 BSC Monitoring Record Form

BSC Monitoring Record Form
OPP Microbiology Laboratory

Date	Init.	BSC#	Downflow (FPM)	Exhaust (CFM)	Use/Comments/Corrective Action*

- Record the actions performed in the BSC whether it be transfers or Use Dilution testing, etc. Also record in this space any alarms (high flow or low flow) that occur during use and the Exhaust (CFM) that triggered the alarm. Document actions taken when BSC fails to conform to manufacturers specifications, e.g., alarm goes off, call for service, etc.